ON THE MECHANISM OF ACTION OF ALDOSTERONE ON SODIUM TRANSPORT: THE ROLE OF RNA SYNTHESIS*

By George A. Porter,† Rita Bogoroch, and Isidore S. Edelman

THE CARDIOVASCULAR RESEARCH INSTITUTE AND THE DEPARTMENTS OF MEDICINE AND PHYSIOLOGY, UNIVERSITY OF CALIFORNIA SCHOOL OF MEDICINE (SAN FRANCISCO)

Communicated by Julius H. Comroe, Jr., September 30, 1964

Crabbé[1] pioneered the use of the isolated urinary bladder of the toad (*Bufo marinus*) in studies on the action of mineralocorticoids on active Na^+ transport. By modifying the experimental conditions we were able to obtain a sensitive and reproducible response to mineralocorticoids *in vitro* in this system and recently proposed that aldosterone and related mineralocorticoids act by enhancing DNA-directed synthesis of RNA resulting in an increased rate of protein synthesis.[2, 3] The newly synthesized proteins may be enzymes involved in the coupling of metabolism to Na^+ transport.[3] This hypothesis was based on the following observations: (1) Uptake of H^3-aldosterone by the isolated toad bladder was complete before the onset of the rise in Na^+ transport. (2) Removal of aldosterone from the bathing media during the latent period had no effect on the magnitude or the time course of the response of the Na^+ transport system. (3) High-resolution radioautography disclosed H^3-aldosterone selectively localized in the nuclei of the epithelial cells of the toad bladder. (4) Actinomycin D and puromycin blocked the action of aldosterone and had little effect on vasopressin-induced stimulation of Na^+ transport. (5) Aldosterone had only a minimal effect on Na^+ transport in substrate-depleted toad bladders, but addition of glucose or pyruvate to the media produced striking potentiation of the mineralocorticoid effect. Specific blockade of the effect of aldosterone on Na^+ transport by actinomycin D was also observed by Williamson[4] for the rat kidney, and by Crabbé and De Weer[5] for isolated toad bladder and skin.

We wish to present additional evidence that aldosterone stimulates nuclear synthesis of RNA, and that RNA synthesis mediates its action on Na^+ transport.

Methods and Results.—(1) *RNA synthesis in epithelial cell preparations (continuous labeling with H^3-uridine):* After double pithing of *Bufo marinus*, the urinary bladder was removed, divided at the cloacal insertion, and rinsed with frog-Ringer's solution (Na^+ = 114 mEq/liter, K^+ = 3.5 mEq/liter, Ca^{++} = 5.4 mEq/liter, Cl^- = 120.4 mEq/liter, HCO_3^- = 2.5 mEq/liter, osmol. = 0.228, and pH in air = 8.4) containing 1.7×10^{-5} *M* mecholyl chloride. The hemibladders were mounted as diaphragms between glass chambers similar to those of Ussing and Zerahn.[6] Residual mecholyl chloride was removed by washing with 30 ml of frog-Ringer's solution for 15 min. All hemibladders were incubated overnight in 40 ml of frog-Ringer's solution containing glucose (10^{-2} *M*), penicillin G (0.1 mg/ml), and streptomycin sulfate (0.1 mg/ml). The following morning, all solutions were replaced with fresh frog-Ringer's solution containing glucose (5.5×10^{-3}*M*) and 20 μC of H^3-uridine (specific activity 2 mC/μM). One hour later d-aldosterone was added to the serosal medium of one hemibladder (final concentration 7×10^{-7} *M*) and an equal volume of diluent to the serosal medium of the control hemibladder. Active Na^+ transport was measured by the short-circuit current (scc) method of Ussing and Zerahn.[6]

Pairs of hemibladders were removed from the chambers at 1, 2, and 4 hr after the addition of aldosterone, and epithelial cells were collected by the method of Hays and Singer.[7] The epithelial cells were homogenized in cold 4 per cent perchloric acid (PCA) and successively washed with 4 per cent PCA, 70 per cent ethanol, 100 per cent ethanol, and anhydrous ether. The precipitate was resuspended in buffer and incubated at 37°C for 1 hr with 50 μg of bovine pancreatic ribonuclease.[8] The RNA content of the supernatant phase was estimated from the 260/280 ratio read in a Zeiss spectrophotometer. Radioactivity was measured by dissolving a 0.5-ml aliquot of supernatant in 15 ml of scintillation solution, and assayed in a liquid scintillation spectrometer.[9] The precipitate that resisted ribonuclease degradation was digested in 1.0 N KOH at 56°C, dissolved in scintillation solution, and assayed for radioactivity as described.

The summary of the results of these experiments is given in Table 1. The specific activity of RNA was 14, 17, and 29 per cent greater in the aldosterone-treated epithelial cells than in the control cells at 1, 2, and 4 hr, respectively. The 2- and 4-hr values are significant at the 2.5 per cent level. At 2 hr the scc ratio was 10 per cent greater and at 4 hr 46 per cent greater in the aldosterone-treated hemibladders than in the control hemibladders (the respective p values were 0.001 and 0.025). Of the total radioactivity in the epithelial cells, only 5 per cent resisted digestion by ribonuclease.

TABLE 1

EFFECT OF ALDOSTERONE ON RNA SYNTHESIS IN SEPARATED EPITHELIAL CELLS OF THE TOAD BLADDER (CONTINUOUS LABELING WITH H^3-URIDINE)

No. of pairs	Time* (hr)	RNA specific activity† (aldosterone/control)	scc Ratio‡ (aldosterone/control)	scc (at time 0)§ (μA/2.54 cm^2)
8	1	1.14 ± 0.14 (0.4)	1.02 ± 0.04 (0.5)	A 128 ± 20 C 112 ± 10
10	2	1.17 ± 0.06 (0.025)	1.10 ± 0.02 (0.001)	A 144 ± 17 C 145 ± 26
9	4	1.29 ± 0.10 (0.025)	1.46 ± 0.18 (0.025)	A 119 ± 10 C 114 ± 16

* Zero time was set at the time of addition of aldosterone to the serosal media.

† The ratio of the specific activity of RNA in cpm/mg of RNA of the aldosterone-treated epithelial cells divided by the specific activity of the paired control. Mean ± s.e.m. p value is given in parentheses.

‡ The "scc ratio" denotes the short-circuit current at time t divided by the short-circuit current at time 0. Mean ± s.e.m. of the scc ratio of the aldosterone-treated hemibladders divided by the scc ratio of the paired control, at the time the hemibladders were removed from the chambers for RNA assay. p value is given in parentheses.

§ Mean ± s.e.m. of the short-circuit current at time 0 of the hemibladders treated with aldosterone (A) and the paired controls (C).

These results indicate that aldosterone increased the rate of incorporation of uridine into epithelial cell RNA and that the effect on uridine incorporation preceded or paralleled the effect on Na^+ transport. Additional experiments were executed to establish whether the increased rate of uridine incorporation preceded the rise in the rate of Na^+ transport.

(2) *RNA synthesis in hemibladders (pulse-labeling with H^3-uridine):* The hemibladders were removed from the toads, mounted, incubated, and monitored as described in (1). d-Aldosterone (final concentration 7×10^{-7} M) was added to the serosal medium of one hemibladder, and the diluent to that of the paired control at time zero. Sixty minutes before the hemibladders were to be removed from the chambers for radioassay, 20 μC of H^3-uridine was added to the serosal media of all hemibladders. The H^3-uridine enriched media were removed 30 min later and re-

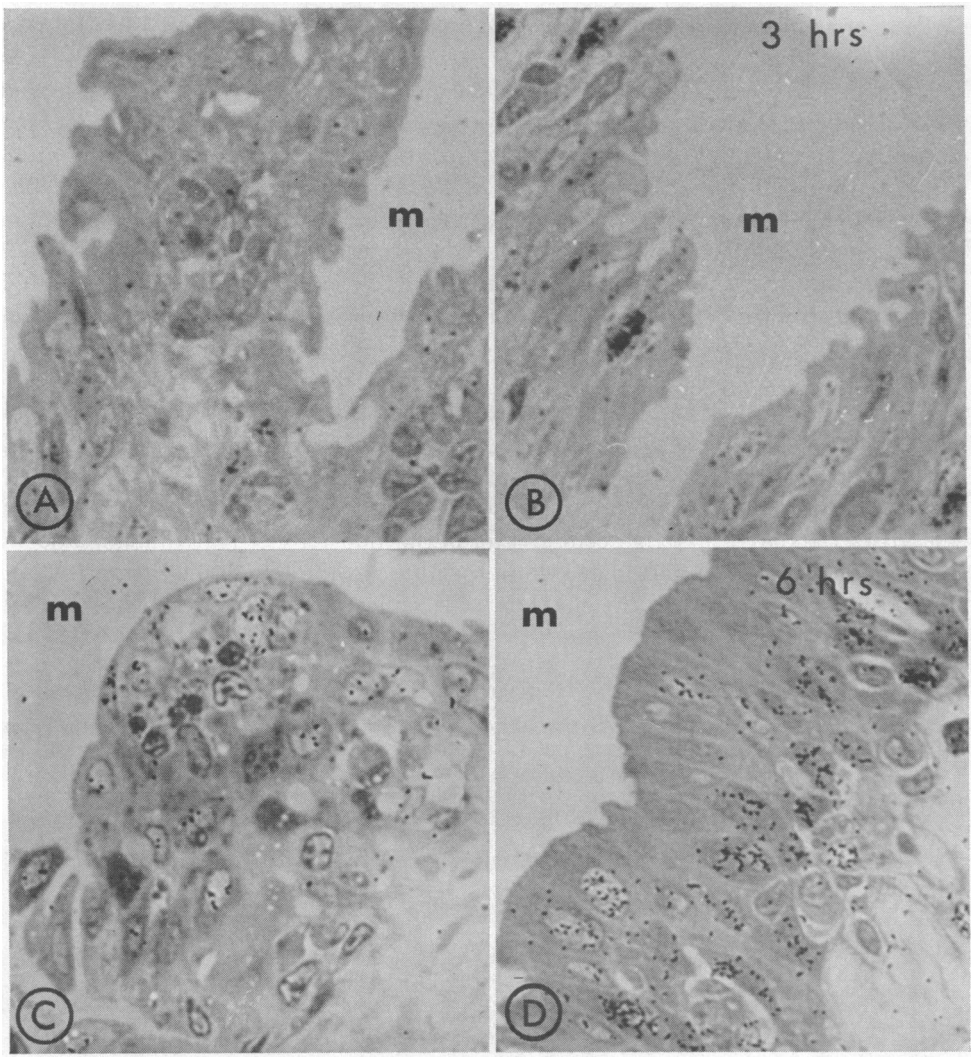

FIG. 1.—Photomicrographs of sections of toad bladder and overlying radioautographs (*Methods* in text). *m* denotes the mucosal surface. (*C*) and (*D*) were taken with phase contrast optics. (*A*) Toad bladder exposed to H^3-uridine for 3 hr and no aldosterone. (*B*) Toad bladder exposed to H^3-uridine and aldosterone for 3 hr. (*C*) Toad bladder exposed to H^3-uridine for 6 hr and no aldosterone. (*D*) Toad bladder exposed to H^3-uridine and aldosterone for 6 hr. Magnification 1024 ×.

placed with fresh frog-Ringer's solution that contained cold uridine at a concentration of 10^{-3} *M*. The hemibladders removed from the chambers 45 min after time zero were exposed to cold uridine for 15 rather than 30 min. Immediately after removal from the chambers, the hemibladders were homogenized in KMT buffer (KCl = 0.14 *M*, $MgCl_2$ = 0.0015 *M*, tris = 0.01 *M*, pH = 7.2) that contained 0.1 gm/100 ml of sodium dodecyl sulfate (recrystallized) and 0.5 ml/100 ml of *n*-butanol. The polynucleotides were extracted in a water:chloroform:amyl alcohol system by the method of Ts'o and Squires.[10] The polynucleotides precipitated from the aqueous phase with ethanol contained 85 per cent of the radioactivity. The pellicle at the

interface between the aqueous and the organic phases contained 15 per cent of the radioactivity. The aqueous-soluble, ethanol-insoluble polynucleotides were redissolved in KMT buffer, treated with ribonuclease, and the specific activity determined as described in (1).

The results of these experiments are given in Table 2. Incorporation of H^3-uridine into RNA was 12, 28, 43, and 30 per cent greater in the aldosterone-treated hemibladders than in the control hemibladders at 0.75, 1.5, 3, and 6 hr, respectively. The 1.5-, 3-, and 6-hr values are significant at the 5 per cent level. At 1.5 hr the rate of Na^+ transport had not yet been raised by aldosterone. Thus, aldosterone stimulated an increased rate of uridine incorporation before the onset of the effect on Na^+ transport.

TABLE 2

EFFECT OF ALDOSTERONE ON RNA SYNTHESIS IN TOAD BLADDERS (PULSE-LABELING WITH H^3-URIDINE)*

No. of pairs	Time (hr)	RNA specific activity (aldosterone/control)	scc Ratio (aldosterone/control)	scc (at time 0) (μA/2.54 cm^2)
11	0.75	1.12 ± 0.10 (0.2)	0.97 ± 0.02 (0.1)	A 176 ± 12 C 166 ± 13
11	1.5	1.28 ± 0.08 (0.025)	0.98 ± 0.03 (0.4)	A 155 ± 14 C 144 ± 17
12	3	1.43 ± 0.21 (0.05)	1.30 ± 0.06 (0.001)	A 140 ± 12 C 130 ± 13
11	6	1.30 ± 0.13 (0.05)	1.74 ± 0.20 (0.005)	A 147 ± 18 C 161 ± 20

* The conventions used in the construction of tnis table are the same as in Table 1.

A suspension of the pellicle residue was treated with ribonuclease in KMT buffer, and the acid-soluble phase was assayed for radioactivity and oligonucleotides. In contrast to the water-soluble RNA, the RNA of the pellicle from the aldosterone-treated hemibladders was not labeled at a significantly higher rate than that of the paired controls; the ratios of the specific activities (aldosterone/controls) were 1.07 ± 0.11 at 0.75 hr, 0.88 ± 0.09 at 1.5 hr, 0.96 ± 0.05 at 3 hr, and 1.21 ± 0.11 at 6 hr. The site of the aldosterone effect was studied by high-resolution radioautography.

(3) *Localization of the site of H^3-uridine incorporation:* Pairs of hemibladders were mounted in glass chambers, incubated, monitored, and treated with H^3-uridine (final concentration 1 μC/ml) and aldosterone or the diluent as described in (1). Pairs of hemibladders were removed from the chambers 1.5, 3, or 6 hr after the addition of aldosterone or the diluent to the media, and fixed in 3.125 per cent glutaraldehyde, stored in cacodylate buffer overnight, postfixed in 1 per cent osmic acid for 1 hr, dehydrated by passage through a graded series of ethanol solutions, and embedded in araldite.[11, 12] Sections 1–1.5 μ thick were cut on a Porter-Blum microtome, coated by dipping in either Ilford K-5, Kodak NTB-2, or NTB-3 liquid emulsion, exposed for 6–120 days, and developed with Kodak D-19 developer. The developed preparations were stained with Kingsley's solution.[3]

As shown in Figure 1, at 3 hr the nuclei of the epithelial cells in the control sections (*A*) were sporadically labeled with H^3-uridine, but many of the nuclei of the aldosterone-treated epithelial cells (*B*) were heavily labeled.

After 6 hr of exposure to aldosterone, all of the nuclei of the epithelial cells (*D*) were heavily labeled with H^3-uridine except for the nuclei of the basal cells. In contrast, the nuclei of the epithelial cells of the control hemibladders (*C*) showed

much less intense labeling, and the basal cells were virtually unlabeled. Although most of the activity was still located in the regions of the nuclei of the epithelial cells, scattered areas of cytoplasmic labeling were evident in the control as well as the aldosterone-treated sections.

The chemical and radioautographic data, therefore, support the concept that aldosterone reacts with a nuclear receptor to increase the rate of synthesis of RNA. Consequently, steroids that have little or no effect on Na^+ transport in the toad bladder system, such as progesterone,[2] may show little or no nuclear accumulation and little or no stimulation of RNA synthesis.

(4) *Localization of H^3-progesterone in toad bladder:* Pairs of hemibladders were mounted, incubated, and monitored as described in (1). At time 0, 50 μC of H^3-progesterone (repurified chromatographically by Dr. Alan Goldfien) was added to each of the serosal solutions with sufficient cold progesterone to give a final concentration of 7×10^{-7} *M*. At 1.5, 3, and 6 hr following the addition of the H^3-progesterone to the media, hemibladders were removed from the chambers, blotted briefly, and immersed in liquid propane (−170°C). Sections 10 μ thick were cut on an International cryostat microtome, and radioautographs were prepared as described.[3]

Representative radioautographs of toad bladders exposed to H^3-progesterone for 1.5 hr (left-hand panel) and for purposes of comparison radioautographs of toad bladders exposed to H^3-aldosterone for 1.5 hr (right-hand panel) are shown in Figure 2. At low magnification, H^3-progesterone was diffusely distributed between the mucosal epithelial layer and the subepithelial layers, whereas H^3-aldosterone, as noted previously, was preferentially localized in the mucosal epithelial layer.[3] At a magnification of 1600 ×, H^3-progesterone was about evenly divided between the nuclear and cytoplasmic areas (left-hand panel) and, in contrast, H^3-aldosterone was preferentially associated with the nuclear areas (right-hand panel).

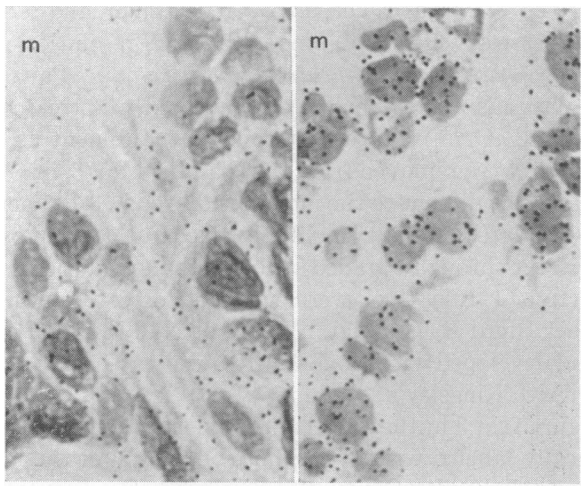

FIG. 2.—Phase contrast photomicrographs of sections of toad bladder and overlying radioautographs (*Methods* in text). Toad bladder exposed to H^3-progesterone for 1.5 hr is shown in the left-hand panel. Toad bladder exposed to H^3-aldosterone for 1.5 hr is shown in the right-hand panel. The magnification was 960 ×. *m* denotes the mucosal surface.

The nuclear receptor for aldosterone appears to have relatively little affinity for progesterone.

(5) *Effect of progesterone on RNA synthesis (pulse-labeling with H^3-uridine):* Pairs of hemibladders were mounted, incubated, monitored, pulsed with H^3-uridine, and the specific activity of the isolated RNA was determined as described in (2). At time 0, progesterone (final concentration 7×10^{-7} *M*) was added to the serosal medium of one hemibladder and the diluent to that of the control. The results given in Table 3 indicate that progesterone had no significant effect on the rate of Na^+ transport and had little or no effect on the initial over-all rate of uridine incorporation. After 3 hr, however, uridine incorporation into RNA was 18 per cent greater in the progesterone-treated hemibladders than in the control hemibladders ($p \cong 0.05$). Nevertheless, the effect of progesterone on RNA synthesis is clearly less than that of aldosterone (cf. Tables 2 and 3).

TABLE 3

EFFECT OF PROGESTERONE ON RNA SYNTHESIS IN TOAD BLADDERS (PULSE-LABELING WITH H^3-URIDINE)*

No. of pairs	Time (hr)	RNA specific activity (progesterone/control)	scc Ratio (progesterone/control)	scc (at time 0) (μA/2.54 cm^2)
9	1.5	0.92 ± 0.07 (0.2)	1.00 ± 0.03 (>0.9)	A 155 ± 20 C 164 ± 9
9	3	1.18 ± 0.09 (~0.05)	0.99 ± 0.06 (0.5)	A 120 ± 17 C 128 ± 11
8	6	1.10 ± 0.13 (0.4)	1.11 ± 0.16 (0.5)	A 101 ± 17 C 87 ± 18

* The conventions used in the construction of this table are the same as in Table 1.

The radioactivity in the pellicle was 15 per cent of the total radioactivity of the extracts. The specific activity of the RNA of the pellicle from the progesterone-treated hemibladders did not differ significantly from that of the paired controls. The ratios of the specific activities (progesterone/controls) were 0.94 ± 0.08 at 1.5 hr, 1.10 ± 0.08 at 3 hr, and 1.04 ± 0.05 at 6 hr.

Since progesterone has a lesser affinity than aldosterone for the nuclear receptor and a lesser effect on RNA synthesis, progesterone should not act as a competitive inhibitor of the action of aldosterone on Na^+ transport at equimolar concentrations.

(6) *Effect of progesterone on the action of aldosterone on Na^+ transport:* Pairs of hemibladders were mounted, incubated, and monitored as described in (1). One hour before adding d-aldosterone to the serosal media of both hemibladders (final concentration = 7×10^{-7} *M*), progesterone was added to the serosal medium of one hemibladder (final concentration = 7×10^{-7} *M*) and an equal volume of diluent to the serosal medium of the control hemibladder.

Neither the latent period nor the subsequent increase in the scc were significantly influenced by the presence of progesterone in the serosal medium (Fig. 3). According to Sharp and Leaf,[13] at a concentration ratio of 540:1 (progesterone = 10^{-4} *M* and d-aldosterone = 1.85×10^{-7} *M*) progesterone antagonized the action of aldosterone on Na^+ transport across the toad bladder. The specificity of this antagonism, however, is questionable since concentrations of progesterone greater than 10^{-5} *M* were toxic to all cells in organ culture.[14]

Discussion.—The concept that steroid hormones exert their physiological and morphological effects through regulation of enzymatic activity has gained consider-

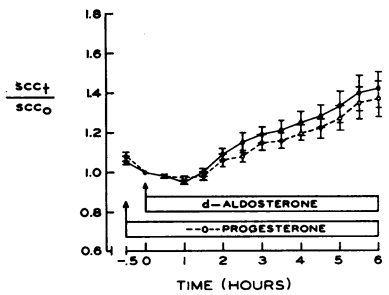

FIG. 3.—Effect of progesterone on the action of aldosterone on Na^+ transport (13 paired experiments). Progesterone (final concentration = $7 \times 10^{-7} M$) was added to the serosal medium of one of the hemibladders (—o—), and d-aldosterone (final concentration = $7 \times 10^{-7} M$) was added to the serosal media of both hemibladders at the times indicated by the arrows. The absolute scc at time zero was 157 ± 12 μA/2.54 cm^2 for the aldosterone group and 155 ± 14 μA/2.54 cm^2 for the aldosterone + progesterone group.

able attention in recent years.[15-17] One mode of steroidal regulation of enzyme activity is induction of *de novo* synthesis of proteins. Especially well-studied examples of this phenomenon are cortisol-induced synthesis of tyrosine transaminase, prednisolone-induced synthesis of glutamic-alanine transaminase, and cortisone-induced synthesis of tryptophan pyrrolase.[18-20] In a number of systems, increased synthesis of nuclear RNA precedes steroidal induction of enzyme synthesis.[20-22] These and other studies have lent credence to the concept of steroidal action via stimulation of DNA-mediated synthesis of messenger RNA,[16] although proof of the messenger hypothesis of steroid action has not yet appeared.[22-24] Feigelson and Feigelson[23] proposed that the cortisone-induced increase in the rate of incorporation of tracers into hepatic RNA is a consequence of an increase in the synthesis of purines secondary to deamination of amino acids rather than a primary effect of the hormone on RNA synthesis.

In the toad bladder system, H^3-aldosterone is preferentially accumulated by the nuclei of the epithelial cells, and actinomycin D and puromycin selectively block mineralocorticoid action.[3] The present study provides additional evidence that aldosterone stimulates Na^+ transport by activating nuclear synthesis of RNA. We found that: (1) Aldosterone enhanced the rate of H^3-uridine incorporation into RNA of the isolated toad bladder before an effect on Na^+ transport was seen. (2) By radioautography, aldosterone increased nuclear incorporation of H^3-uridine in the effector epithelial cells. (3) H^3-progesterone was not preferentially associated with the epithelial nuclei. (4) Progesterone did not stimulate an increased rate of Na^+ transport or an increased rate of H^3-uridine incorporation into RNA. (5) As predicted, progesterone, at equimolar concentrations, did not inhibit the action of aldosterone on Na^+ transport.

Summary.—Isotopic incorporation, radioautographic, and physiological evidence of the mediating role of *de novo* synthesis of nuclear RNA in the action of aldosterone on Na^+ transport *in vitro* has been presented. The failure of progesterone, a steroid with little effect on active Na^+ transport across the toad bladder, to associate preferentially with the epithelial nuclei suggests that physiological specificity of steroids may depend on the affinity of the steroid for specific nuclear (? chromosomal) receptors.

The authors were ably assisted by Ella Highland and Jennifer Scott throughout this study.

* Support for this research was provided by the USPHS-NIH (grant HE-06285) and the Clough and Breon Memorial Funds of the University of California, San Francisco.

† Special Fellow of the USPHS.

[1] Crabbé, J., *Presses Acad. Européennes S.C.* (1963).

[2] Porter, G. A., and I. S. Edelman, *J. Clin. Invest.*, **43**, 611 (1964).
[3] Edelman, I. S., R. Bogoroch, and G. A. Porter, these PROCEEDINGS, **50**, 1169 (1963).
[4] Williamson, H. E., *Biochem. Pharmacol.*, **12**, 1449 (1963).
[5] Crabbé, J., and P. De Weer, *Nature*, **202**, 298 (1964).
[6] Ussing, H. H., and K. Zerahn, *Acta Physiol. Scand.*, **23**, 110 (1951).
[7] Hays, R. M., and B. Singer, *Federation Proc.*, **22**, 623 (1963).
[8] Nutritional Biochemical Corp., Cleveland, Ohio.
[9] Packard Instrument Corp., La Grange, Ill.
[10] Ts'o, P. O. P., and R. Squires, *Federation Proc.*, **18**, 341 (1959).
[11] Sabatini, D. D., K. G. Bensch, and R. Barrnett, *J. Cell. Biol.*, **17**, 19 (1963).
[12] Luft, J. H., *J. Biophys. Biochem. Cytol.*, **9**, 409 (1961).
[13] Sharp, G. W. G., and A. Leaf, *Nature*, **202**, 1185 (1964).
[14] Rivera, E. M., J. J. Elias, H. A. Bern, N. P. Napalkov, and D. Pitelka, *J. Natl. Cancer Inst.*, **31**, 671 (1963).
[15] Knox, W. E., V. H. Auerbach, and E. C. C. Lin, *Physiol. Rev.*, **36**, 164 (1956).
[16] Karlson, P., *Perspectives Biol. Med.*, **6**, 203 (1963).
[17] Jervell, K. F., *Acta Endocrinol.*, **44**, 3 (1963).
[18] Kenney, F. T., *J. Biol. Chem.*, **237**, 3495 (1962).
[19] Segal, H. L., and Y. S. Kim, these PROCEEDINGS, **50**, 912 (1963).
[20] Feigelson, P., M. Feigelson, and O. Greengard, *Recent Progr. Hormone Res.*, **18**, 491 (1962).
[21] Liao, S., and H. G. Williams-Ashman, these PROCEEDINGS, **48**, 1956 (1962).
[22] Kenney, F. T., and F. J. Kull, these PROCEEDINGS, **50**, 493 (1963).
[23] Feigelson, P., and M. Feigelson, *J. Biol. Chem.*, **238**, 1073 (1963).
[24] Garren, L. D., R. R. Howell, and G. M. Tomkins, *J. Mol. Biol.*, **9**, 100 (1964).

INDUCTION BY ADENOVIRUS TYPE 7 OF TUMORS IN HAMSTERS HAVING THE ANTIGENIC CHARACTERISTICS OF SV40 VIRUS

BY R. J. HUEBNER,* R. M. CHANOCK,* B. A. RUBIN,† AND M. J. CASEY

NATIONAL INSTITUTES OF HEALTH, BETHESDA, MARYLAND

Communicated October 14, 1964

Tumors having the virus-specific antigenic characteristics of those produced by SV40 virus[1-5] developed in 27 of 36 hamsters injected as newborns with adenovirus type 7, strain L. L., a strain isolated and grown continuously in monkey kidney tissue cultures. The antigenic character of these tumors was particularly interesting because the 28th passage inoculum which produced them contained no detectable SV40 virus, the latter having been eliminated from the L. L. strain at 23rd passage with the use of hyperimmune SV40 monkey serum.

The SV40 tumor antigens were demonstrated in the complement fixation (CF) test with the use of serums from tumorous hamsters which contained virus-specific antibodies to SV40 tumor[2] and to similar cell-associated antigens found in cells infected with SV40 virus;[6, 7] conversely, CF antibodies to SV40 tumor and the cell-associated antigens were demonstrated in the serums of hamsters carrying the L. L.-induced tumors.

Shortly after these findings were available, Rowe and Baum, and Rapp *et al.* (see accompanying papers in this issue of these PROCEEDINGS[8, 9]), using fluorescent antibody techniques, demonstrated similar virus-specific antigens in various tissue cultures infected with adenovirus 7, strain L. L.